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THE DENGUE: A STUDY OF ITS PATHOLOGY AND MODE OF PROPAGATION.

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ALTHOUGH the literature of the Dengue is quite voluminous, and a large number of valuable reports have been made of different epidemics, from that of Java, Cairo and Alexandria in 1779, to that of the eastern end of the Mediterranean in 1889, yet nothing definite has been ascertained with regard either to its mode of spreading or its pathology. A considerable number of autopsies have been performed by careful observers, and no definite anatomical lesions have been found. At one time it was considered a purely miasmatic infection, but since the epidemic of 1899 a strong opinion has prevailed among those who have given

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the matter careful study that the disease is purely contagious.

IS DENGUE CONTAGIOUS ?

Professor Dr. O. Leichtenstern, of Cologne, Nothnagel's "*Specielle Pathologie und Therapie*," iv., Band 1, sums up a very careful study of the whole literature by saying that it belongs to "*den contagiösmiasmatischen Krankheiten*." In other words, he believes that it is contagious, but that the contagion requires for its propagation a certain miasmatic environment, and without this it cannot be propagated. The rapidity with which it spreads from one house to another after entering a city, and from one member of a household to another, having once entered the family, shows that it is contagious (von Düring, "*Denguefieber in Constantinople*," *Monatschrift für Praktische Dermatologie*, 1890, 2 S., p. 16; Malé, article "Dengue," *Diction. encyclop. des Sciences Med.*; Sulzer, Mordtmann, Skottowe, and others). On the other hand, the inability of the disease to spread when introduced into other regions than those in which the miasmatic conditions are favourable have forced many accurate observers in the East and in America to the conclusion that it was not a contagious but a miasmatic disease (S. Ornstein, "*Zur Dengue-Frage*," *Deutsche Med. Wochenschr.*, 1890, 2; Wernich, Eulenberg's "*Realencyklopädie*, 1886, v.); Chrysococho in Smyrna, A. Hirsch, and others. There is a great multiplicity of evidence to show that it does not spread to any extent when carried away from certain low-lying regions which are its favourite abode. Epidemics in Cuba, Jamaica, East Indies, Réunion, Martinique, and Madagascar, all seem to show that in spite of the most active communication, the dengue cannot spread to any extent when carried into dry and high places in the interior. Very frequently people come down to the coast from the interior, contract the germs of the disease, and then return home, there to be smitten with the fever without the other inmates of the household catching it. This seeming lack of contagiousness under certain conditions and extreme contagiousness under certain other conditions up to the present time has remained unexplained.

With the view of finding out what the conditions were which rendered this disease contagious at one time and not at another, the epidemic which occurred in the city of Beyrouth in the summer of 1901 was seized upon as an opportunity for careful experiment and study of the whole question. This city has certain marked natural advantages which render a study of the dengue easy and enable a student to carry out certain experiments otherwise well nigh impossible. It is situated on the sea-shore, with the Lebanon range rising very abruptly from its outskirts to a height of from 2,000 to 9,000 feet; so that in an hour's or two hours' time one may observe the disease at the level of the sea or several thousand feet above that level. Early in July of 1901 the epidemic began, at first slowly, but as it increased it seemed to gain force and virulence, spreading through every part of the city and the outlying villages. During this epidemic, which was a very severe one, few houses in the city escaped, and usually not many members of a household were spared when the disease once found an entrance. The epidemic was

a typical one, the fever lasting from three to eight days, and during defervescence producing an eruption more or less marked. A considerable proportion of the cases—in my own practice 30 per cent.—were followed by a second attack, usually lighter than the first, after an interval of four to fifteen days. During the interval the patient almost always complained of not being entirely well. In a few cases I found three distinct attacks, each one very severe in character, and each one accompanied by an eruption, with considerable desquamation. A description of the 500 and some odd cases that I have seen during the outbreak would differ in no particular way from the cases described by Manson and others.

IS THE MOSQUITO THE FACTOR ?

I saw something of the epidemic which prevailed on the Syrian coast in 1889, and my experience gained at that time, together with what I had learned from the reports of other observers in different epidemics, inclined me to the belief that in the mosquito was to be found the factor which made the dengue in certain localities very contagious, while not at all in others. Between Beyrouth and the mountains to the south exists a immense sand-bank; to the north irrigated mulberry orchards. The houses in the city are without exception badly infested with mosquitoes. With but few exceptions the villages dotting the slopes of Lebanon contain them in greater or less numbers. In the city of Beyrouth itself none of the forms of *Anopheles* have been found, although careful search has been made for them by others as well as myself. The city, however, is terribly infested with *Culex fatigans* and *Stegomyia fasciata*. I have searched carefully a considerable number of the villages on the slopes of Lebanon facing the city, and have found the *Culex fatigans* in almost all of them, even to a height of 5,000 feet above the sea-level. I have also found *Stegomyia fasciata*, although less frequently.

EXPERIMENTS WITH CULEX FATIGANS.

At the beginning of the epidemic I commenced a series of experiments in order to determine, if possible, whether the *Culex fatigans*, which exists in perfect swarms in the city, could carry the dengue from person to person.

(1) The first experiment was done with a mother and her nursing child. The day the mother was taken with the initial chill all the mosquitoes in one room were killed by generating chlorine gas, and each day after she entered another room in which the mosquitoes had been so killed. During the fifteen days' time she was under observation three rooms were used, the room after being freed from mosquitoes being each time protected as well as possible from their re-entrance. The mother, who was extremely anxious to protect her son of 8 months from the disease, submitted to the annoyance and trouble. The child was allowed to nurse from his mother during the whole attack of the fever, which was a severe and typical one, and escaped any observable form of infection.

(2) Experiment No. 2 was carried out in the same way, and exactly the same precautions were taken to

keep out the mosquitoes. There were four small children who slept in the same bed on the floor, their ages ranging from 4 to 11 years. The eldest of the four contracted the fever and the other three played in the same room where he lay sick and slept in the same bed with him at night, no one of the three contracting the disease. The rooms were kept free from mosquitoes during thirteen days.

(3) In the third experiment the father of three children contracted the disease in the city, and was willing to submit to almost any inconvenience to protect his wife and children. The same precautions were taken as in Nos. 1 and 2. The man had a severe attack lasting five days, accompanied by high fever, eruption and desquamation. The rooms were kept as free as possible from mosquitoes during seventeen days, with the result that no other member of the family was taken ill.

In several other cases I tried the same experiment, but gave it up because of carelessness on the part of the household, which resulted in my finding mosquitoes in the rooms when I made my visit of inspection. They said the dengue was better than the confinement. However, in those cases where care was taken the results were all that could be expected.

DIRECT INOCULATION.

The evidence, however, in these experiments was of a negative kind, and in order to obtain something more positive, several experiments were done in direct inoculation or infection. My excuse for exposing men to infection was that everyone in the city not immune to the disease was going to get it anyway, also that the disease was entirely without danger to life in young and healthy men. The character of the experiment was explained in each case, and the young man, for only young men were used, expressed his entire willingness to have the "aboo-rikal," as it is called, for the sake of the money offered. The difficulty was not to find one willing to undergo the experiment, but to select the best ones from the many who offered. Four men were selected, inmates of houses in which no case of dengue had as yet occurred. In each case several mosquitoes were taken from the netting of patients sick with the fever and put inside the netting of the candidate for the disease, he sleeping night after night with the mosquito in the netting. In one case the initial chill came on five days after the mosquitoes were put in, six days afterwards in a second, and four days after in the third. In the fourth case, no attack having occurred at the end of a week, another batch of infected mosquitoes were put in, with a negative result. This young man gave the history of having had a severe attack of dengue accompanied by eruption and desquamation during the epidemic of 1889. Possibly he was immune for that reason. During that epidemic I had a very severe attack, and during this last one I escaped although constantly exposed, and although I took no means to protect myself. In all of these cases the men were under agreement to remain in their houses in order not to contract the disease in any other way. In a city where so infectious a disease was raging I still felt that these three men might have received the contagion in

some other way. In order to avoid this objection mosquitoes were taken from inside the netting of a dengue patient and carried up to a village on the mountain slope where as yet no case had occurred. After visiting my patient for the infected mosquitoes I changed my clothing and took a bath before mounting my horse to ascend the mountain. In this village, about 3,000 feet above the sea, there are almost no mosquitoes. It is dry and very healthy. I easily found two young men in different parts of the village who consented for a consideration to take their chances of having the dengue. One of them, after sleeping four nights under the netting with the mosquitoes, was taken with a severe typical attack of dengue. The other had his initial chill after having passed five nights in the company of the mosquitoes. These men continued to sleep under the mosquito nettings I had prepared for them until some time after they were well, all the mosquitoes in the netting having been killed to avoid infection of other people. No other cases of dengue occurred in this village during the summer that I could learn of, although I made the most careful inquiry.

A considerable number of cases occurred in the large village of Aley, which is 2,300 feet above the sea-level. Few of the houses in this village are free from the *Culex fatigans*. Bhamdoun is about 4,000 feet high and very dry, with but few mosquitoes. Here, although the village is on the line of railway and several cases were imported from Beyrouth, yet the disease did not spread among those who did not descend to the city. Several cases occurred in Ainsofar, which is higher up (about 5,000 feet), but which is not so dry as Bhamdoun and which is more infected by mosquitoes. The villages like Arayeh, Schweifat, &c., lower down and near Beyrouth, suffered almost as badly as Beyrouth. In the beginning of the epidemic of 1889, I passed one night in Beyrouth and then went up to a cabin in the Taurus range, 4,600 feet above the level of the sea. There were twelve of us sleeping in this cabin, which is among the pine trees, and absolutely free from any species of mosquito. On the sixth night after leaving Beyrouth I was attacked by a severe dengue of five days' duration, during which time my temperature ranged from 103° to 105° F. There was an eruption, and I desquamated like a scarlatina case. The other eleven who continued to inhabit the cabin with me remained entirely free from the disease.

It seems to me both from the mass of evidence collected on the character of dengue epidemics by others, and from the experiments which I have described, that the mosquito is undoubtedly the factor which we have considered as the miasmatic one in the spreading of this disease. It seems to me also equally certain that the *Culex* which we have considered up to this time harmless does carry the infection of the dengue. The experiments first described in this article by which others were prevented from taking the disease, although in daily and hourly contact with it, by keeping all mosquitoes away from the infected person, would also show that failing mosquitoes to carry the poison the disease is not contagious.

BLOOD EXAMINATIONS.

Having determined to my own satisfaction that the

mosquito was the means by which the disease was carried from one person to another, the natural conclusion was that the cause of the disease or the virus must be found in the blood. In the hope of finding it there, I examined the blood of over 100 cases of dengue. I must say that I chose for examination only those in which the fever was high, the constitutional symptoms well marked, and the course of the disease typical. The lighter cases, lasting only one or two days, and with a temperature at no time higher than 100° F., were not examined. The methods used in preparing and examining specimens have been the same as for blood containing malaria parasites. The most satisfactory results were obtained by examining the blood fresh. Specimens were prepared according to Ehrlich's method and stained in methylene blue, borax methylene blue, and Ehrlich's blood stain, but the results were disappointing even with the greatest care. Scrupulously clean slides and cover-glasses were carried by me in a Petrie dish to the bedside of every patient I visited, and after the finger-tip was well cleaned with alcohol and ether, the specimen was made and the cover-glass rimmed with vaseline in order to prevent, as long as possible, changes in the corpuscles. Several times the blood was drawn from some of the brightest spots of the eruption, but in these cases the resulting field differed in no way from that of the blood from the finger-tip. The specimens were examined with a Zeiss 2 mm. homogenous immersion lens (aperture 1.40) and either No. 6 or 8 compensation oculars, apochromatic system.

AN ORGANISM PRESENT.

After careful examination of a certain number of specimens I was able to detect in the red blood corpuscles an organism with amœboid movement, and in many ways resembling the *Plasmodium malariae*. The different phases of its existence have, however, been very difficult to follow, since it comes more slowly to maturity, and runs through its cycle in so much longer time, that no matter how much care was taken to keep my specimens, the corpuscles had degenerated before the parasite could be followed along much of its life's phase. The fever lasts in these cases from four to eight or ten days, and in order to see the parasite in different stages, an attempt had to be made to get my specimens prepared in series, as it were, to show the organism at different times from the initial chill to the end of the fever. The material, however, from which to choose specimens was so plentiful that this was not difficult. In each case a record was kept of the day and hour when the initial chill took place, and of the day and hour when the blood was taken. The illustrations are drawings made from specimens prepared in this way and put as nearly in their natural order as possible. After a certain amount of practice and familiarity with the organism I never failed to find it in any case where the exigencies of my practice did not make me leave my work before I had made sufficiently long or patient search.

Fig. 1 is a fairly accurate representation of what I found in a certain number of red corpuscles when the blood was examined within the first twenty-four hours of the attack. The parasite is of a lighter colour than the rest of the corpuscles. It is sometimes seen in the

centre and sometimes at the margin, but constantly changing its position in the corpuscle. It is to be found in a proportionately much smaller number of corpuscles than the malaria parasite in an ordinary case of tertian fever, but it may always be found if careful search is made. In fig. 2 the parasite is shown in a later phase

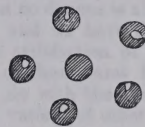


FIG. 1.—A red corpuscle during the first twenty-four hours.

of its cycle, and this is the form in which it is usually seen. It is more easily detected at this time, and it seems to remain in about this state till towards the end of the fever. Another reason why it is more difficult to find than malaria parasites is on account of the absence of pigment such as is formed by the *Plasmodium malariae* and which catches the eye under the microscope even more readily than the parasite itself.

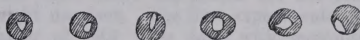


FIG. 2.—A red corpuscle at the end of thirty-six hours, as seen at different times in the field.

THE ORGANISM AN UNPIGMENTED PROTOZOON.

It is evidently a protozoon, but without pigment, and in this it differs from the *Plasmodium malariae*. It is true that Marchiafava and Celli have discovered and carefully described an unpigmented quotidian parasite, but it differs so in its life-history that it need not for a moment be confounded with this. I know of no blood parasite which resembles it so much as the *Pirosoma bigeminum* (*Boophilus bovis*). It, like the *Pirosoma*, is mostly ovoid in form and very frequently

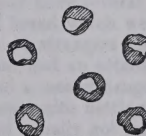


FIG. 3.

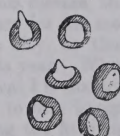


FIG. 4.

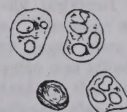


FIG. 5.—A red blood corpuscle and three white ones, large, and with spores of the hæmatozoon in their interior.

shows a sharply pointed extremity as seen in the parasite of Texas fever. Both are unpigmented forms, but the hæmatozoon of the dengue has not the divided form which has given the name *bigeminum* to the *Pirosoma*.

In figs. 3 and 4 the parasite is seen still further advanced, and about the fourth to the sixth day many portions of broken corpuscles are seen scattered throughout the field. Frequently in specimens of this date the hæmatozoon is found pushing its way out from the remnants of a corpuscle, or it is seen with the mere outline of a corpuscle clinging around it. Very soon after its escape it is seen to undergo degeneration. I have never seen a white corpuscle attack and swallow a parasite while the latter was undergoing amoeboid motion, but I have frequently seen degenerated forms or those in which motion, as nearly as I could make out, had ceased, which were engulfed and taken inside active white corpuscles. Frequently towards the end of the attack and for one or two days after the temperature has dropped to normal, leucocytes are found in the field swollen up and markedly enlarged with remnants of the hæmatozoon they have gathered up from the blood plasma.

The blood in cases of second attacks was examined with more than usual care and also in one case of a third attack. In all of them I was able to find this same hæmatozoon. Since the parasites are fewer in number, unaccompanied by pigment, and slower in their development, they are more difficult to find than malaria parasites, but anyone who has had experience in examining for malaria parasites will, I am sure, always find them with care and patience. The constant presence of this hæmatozoon in the red blood corpuscles of those suffering from the dengue, its resemblance to other forms of sporozoa which have been proved to cause fevers such as Texas and malarial fevers, and its propagation from person to person by means of the mosquito, all seem to me to justify the conclusion that in this parasite we have the cause of the dengue fever.

Beyrouth families may be divided into two classes, those who pass the summer in the mountains and those who pass this season in Beyrouth. During the hot months of this summer of 1901 the epidemic raged in the city and left hardly a susceptible person. When it was over and the weather had grown cooler the families who had passed their summer in the mountains came back to the city, believing themselves safe. An epidemic at once broke out among these which did not cease till the commencement of December, and then only apparently because all susceptible individuals had had it. This would seem to show, together with the high percentage of the population attacked (75 to 81 per cent.), that the epidemic ceases not so much because of the approach of cold weather as because of the fact that all individuals not immune have had it. In the month of January, 1902, I had under my care four cases of the dengue, and in February three, in all of which the individuals recently came from the interior where dengue had never been known, and in all of which the diagnosis was made early in the disease by finding the hæmatozoon. None of the seven had been more than a few days in Beyrouth before falling ill with the dengue. These cases show that the dengue may still hang round in some form or other for a considerable length of time after the epidemic ceases. In all the cases cited above and those from which material was taken for experimental purposes, only typical

dengue cases were selected. We all know how convenient a term in our climes "aboo-rikal" is. In every case of fever in which it is not possible to make a diagnosis we can satisfy our patients' friends for the time being, if not our own consciences, by responding that it is the dengue, and this at all times of the year, whether an epidemic is prevailing or not. If it happens to be in the winter when no dengue is about we can say "Aboo-rikal el shita," and we have then named the patient's illness to the satisfaction of his friends. A very large proportion of these cases I am convinced have nothing at all to do with the dengue and ought to be included under a variety of other headings.

The experiments above cited have to my mind cleared up the differences of opinion expressed by different medical writers. Von Düring, Mordtmann, de Brun, Skottowe, and others claiming that it is a highly contagious disease on the one hand, and on the other, Ornstein, Wernich, Chrysoschoos, Hirsch and others that it is a miasmatic one and not contagious at high levels. The observations of those who have found it always contagious have been made in regions where either the *Culex fatigans* or *Stegomyia fasciata* abound, and those of the others where these mosquitoes are not found or in but slight numbers. The first experiments done in the summer of 1901 were done with mosquitoes collected at hazard, and although most of the mosquitoes were *Culices*, yet in almost, if not every case, *Stegomyia* were among them.

THE PHASES OF THE PARASITE IN THE MOSQUITO.

In the summer of 1902 I began a series of experiments in an attempt to follow out the parasite through the different phases of its life in the body of the mosquito. These experiments were done almost wholly with the *Culex fatigans*, which I found in every way, by reason of its habits, voracity, &c., a much more facile insect with which to experiment. During this summer I found considerable difficulty in finding typical dengue cases, and must say, in spite of the well-known fact that a person may have several attacks of this disease, that the cases I found which were typical were for the most part those of strangers coming to the city, or those who had not had an attack the previous year. Whenever I found a case of dengue at all typical I introduced inside his mosquito netting from thirty to fifty hungry *Culices*, and usually got from the netting the following morning one-third or one-fourth of that number with stomachs well filled with blood. These were put in a cage which was liberally provided with water and banana peelings or grapes. The cage was then labelled with date, &c. As many cages were kept as I had cases. Every day a mosquito was killed and with much pains and labour the contents of the stomach, the walls of the same, and the salivary glands were examined under both low and high power. The walls of the stomach and the salivary glands had to be dissected out under the microscope, involving much time and work. The blood of the stomach, which was examined in a normal saline solution, was found to contain the parasite. I observed the changes in its metamorphosis in much the same way and manner as I had found in the blood directly drawn from the patient in the summer

of 1901. It has seemed to me, however, that the development of the parasite in the stomach of the mosquito was more rapid and more distinct than in the blood in the body of the patient. I never failed in any of these cases to find the parasite up to the fifth day after the mosquito sucked the blood, and it has seemed to me that the parasite was detected with more ease and certainty in the blood from the stomach of the mosquito than in that directly from the vessels of the patient. Cases in which the mosquitoes drew the blood early in the attack took more time to develop full-grown spores in the mosquito stomach than those in which the blood was sucked later. However, the changes in the parasite from day to day in the blood drawn from the mosquitoes' stomachs were found to differ in no essential way from those which occurred in the blood of the man sick with the dengue. A much more difficult task was that of the examination of the walls of the stomach. Here I searched carefully from day to day for encysted zygotes, which enlarging should discharge their sporozoites into the general cavity after the manner so beautifully worked out and described by Ross in malaria, but in vain. Neither was I able at any time to discover anything resembling a fusion of a microgamete with a macrogamete in the blood while in the stomach of the mosquito or in the blood of the patient.

SPORULATION.

The evolution of the parasite resembles that described by Lignieres of the *Pirosoma bigeminum*, which it also most resembles in form. By the process of sporulation, in a short time the walls of the stomach and also those of the salivary gland are soon filled in here and there with spores which are for the most part oval, although many are spherical. In mosquitoes which had drawn blood from a patient ill on the fourth day, I was able to find these spores in among the cells of the salivary glands in forty-eight hours' time, showing the possibility of a mosquito's power to infect in a very short time after drawing blood from a dengue patient. The number of spores discharged from the capsule in one parasite differed in different cases. It was only a very short time after their escape from their parent capsules before they were to be found in the walls of the stomach and among the cells of the salivary glands. These spores, I have no doubt, are what are introduced with the saliva of the mosquito in sucking blood and which, developing in the human blood-current, cause the dengue. Mrs. Graham undertook one day to procure a mosquito for me from one of the cages, and while holding the killing bottle over the mosquito to be caught, did not notice until too late that she was being bitten by another. The third day after she was taken to bed with severe nausea and vomiting and high fever, the attack running the course of a typical sharp attack of dengue. This was her first attack of dengue, and there had occurred at no time during the summer any cases in the house, neither had she been anywhere else to expose herself to this disease. The mosquitoes in this cage had drawn blood from the dengue patient fifteen days previous to the time when she was bitten. Some of the mosquitoes in my cages I succeeded in

keeping alive for a month after they had become infected, and even up to that time the spores of this hæmatozoon were found in the salivary glands.

INOCULATION OF DENGUE.

¶ An attempt was made to inoculate individuals with the salivary glands carefully dissected out and thoroughly mixed in a warm sterilised normal peptonised saline solution. This solution was injected under the skin and was taken from a mosquito with a twenty-seven days old infection. On the third day the patient had a chill and high fever, and an attack resembling in every way that of dengue, but so strong that I desisted from further experiments in that line. That this was not septicæmia was proved by finding numerous dengue parasites in the blood. I have also begun a series of experiments in an attempt to find out whether the ova of the mosquito may contain these spores as in the case of the cow tick's ova, which may carry the spores of the *Piroplasma bigeminum*. Smith and Kilborne proved that these latter, when mature, inoculate cattle with Texas fever. As yet, however, I have not been able to settle this point.

In the *British Medical Journal* of July 12th, 1902, may be found a well-written article on the dengue by Dr. F. O. Stedman, of Hong Kong. He says, among other things, "In several cases in which I have examined the blood during the relapse I have found malaria parasites, and I hear that others have had the same experience; thus it seems that the attack of dengue either wakes up malaria, which has been lying dormant in the system, or else renders the patient particularly non-resistant to a chance fresh infection." I believe that Dr. Stedman saw not malaria parasites but the hæmatozoa of the dengue. It is true that it is found in much larger numbers during relapses than in the first attack, but a careful examination will reveal its presence also in the original or first attack. The great mass of people who have a relapse from an attack of dengue have never had any malaria fever either before or after their attack of dengue, and the mass of them live in places (I speak of Syria) where no *Anopheles* are present to infect them with malaria. The parasite, it is true, resembles in the field of the microscope that of the æstivo-autumnal fevers, yet its development in the stomach and body of the mosquito, its phases of life and progress, are too different to be confounded with the unpigmented quotidian parasite of Marchiafava and Celli. While I am not in a position to speak with authority on the kinds of mosquitoes found in Hong Kong, I cannot help but believe that the parasites seen by Dr. Stedman were the same as those described by me in this article, and that long and careful examination will prove them to follow the same life development.

The discovery of the cause of dengue proves that it is also one of the malarial fevers, and therefore the same means now used to prevent malarial fevers must also be used to prevent communities from getting the dengue. It is therefore a question of war against the mosquitoes, and when the *Culex fatigans* has been destroyed from among us I believe the dengue will be with us a thing of the past.



